The Peptide-GPCR Project: matching *C. elegans* neuropeptides to their cognate receptors

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Neuropeptides modulate neural circuits driving adaptive behaviors and key physiological processes such as reproductive behavior, metabolism, feeding, locomotion, and experience-based learning. With over 250 predicted sequences, bioactive peptides are by far the largest and most diverse group of neuromodulatory substances in the *C. elegans* nervous system (Li and Kim, 2010 PMID: 21189676; Bargmann 2012, PMID: 22396302). Most of them are thought to function through the activation of G protein-coupled receptors (GPCRs) belonging to the Rhodopsin and Secretin classes. The *C. elegans* genome encodes at least 120 genes for neuropeptide precursors (FLP, NLP, and INS families), and recent studies have estimated a similar number of about 100 to 150 peptide GPCR genes (Frooninckx et al., 2012 PMID: 23267347; Hobert, 2013 PMID: 24081909). Although peptide signaling has undoubtedly diversified in the nematode lineage, many neuropeptidergic signaling systems share homology to vertebrate and protostomian systems (Mirabeau and Joly, 2013 PMID: 23671109). Growing evidence implicates neuropeptides in many, if not all, behaviors in *C. elegans*. In contrast, only 23 *C. elegans* peptide GPCRs (isoforms included) have been deorphanized so far (Frooninckx et al., 2012 PMID: 23267347), leaving more than 80 percent of all putative peptide receptors with unknown ligands.

Knowledge on functionally active neuropeptide-receptor couples and GPCR affinity is crucial to further expand our understanding of how neuropeptides function and modulate neural circuits. We are therefore undertaking a large-scale deorphanization initiative that aims at matching all predicted peptide GPCRs to their cognate neuropeptide ligand(s). The Peptide-GPCR project builds further on our combined reverse pharmacology approach (Figure 1), which in the past has enabled us to uncover several *C. elegans* neuropeptide systems – including cholecystokinin, pigment dispersing factor, and vasopressin/oxytocin related signaling, among others (Beets et al., 2012 PMID: 23112336; and reviewed in Frooninckx et al., PMID: 23267347). Both knowledge on natural peptides in tissue extracts and *in silico* peptide predictions are used to maximize the chances to identify a receptor's cognate ligand(s). Through this combined approach, GPCRs are challenged with a collection of more than 260 peptides belonging to the established FLP and NLP families. The *in vitro* calcium mobilization strategy allows screening of these hundreds of peptides on all putative peptide GPCRs in a high-throughput manner.

The deorphanization of predicted *C. elegans* peptide GPCRs should provide a basis for furthering our understanding of nematode neuropeptide signaling and modulation. We aim to share progress on newly identified peptide-GPCR couples to the worm community, as we move down the random list of peptide GPCRs in the course of this project. We are working on a project's website through which you can already help us prioritize peptides or receptor candidates. Community members are invited to steer the project's progression via http://worm.peptide-gpcr.org.

References

Bargmann CI (2012). Beyond the connectome: how neuromodulators shape neural circuits. Bioessays 34, 458-465.
PMID: 22396302

Beets I, Janssen T, Meelkop E, Temmerman L, Suetens N, Rademakers S, Jansen G, and Schoofs L (2012). Vasopressin/oxytocin-related signaling regulates gustatory associative learning in *C. elegans*. Science 338, 543-545.
PMID: 23112336

Frooninckx L, Van Rompay L, Temmerman L, Van Sinay E, Beets I, Janssen T, Husson SJ, and Schoofs L (2012). Neuropeptide GPCRs in *C. elegans*. Front. Endocrinol. (Lausanne) 3: 167, 1-18.
PMID: 23267347

Hobert O (2013). The neuronal genome of *Caenorhabditis elegans*. Wormbook 13:1-106 doi: 10.1895/wormbook.1.161.1.
PMID: 24081909

Li C and Kim K (2010). Neuropeptide gene families in *Caenorhabditis elegans*. Adv. Exp. Med. Biol. 692, 98-137.
PMID: 21189676

Mirabeau O and Joly JS (2013). Molecular evolution of peptidergic signaling systems in bilaterians. Proc. Natl. Acad. Sci. U.S.A 110, E2028-2037.
PMID: 23671109

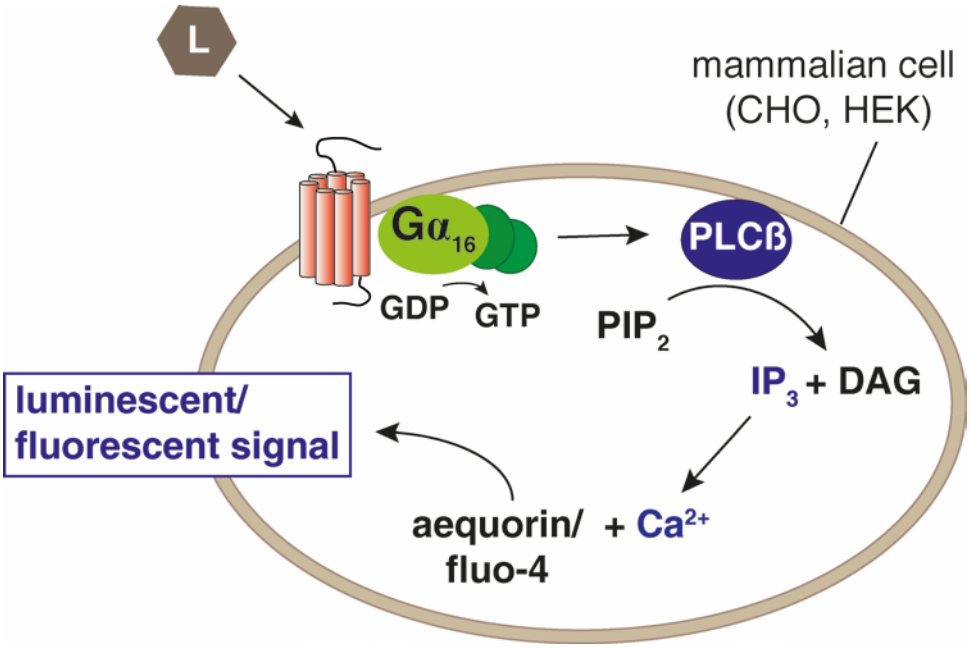

Figure 1. Overview of our deorphanization approach. GPCRs are cloned and heterologously expressed in Chinese hamster ovary (CHO-K1) cells and/or human embryonic kidney (HEK293T) cells co-expressing the $G\alpha_{16}$ protein. This promiscuous Gα subunit can direct

intracellular signaling of most GPCRs to a calcium flux, regardless of the native signaling pathway in endogenous settings. Dose-dependent calcium responses upon GPCR activation are monitored using fluorescent (Fluo-4) or bioluminescent (aequorin) calcium indicators.